EUROPEAN NEURO MUSCULAR CENTRE

ENMC WORKSHOP APPLICATION

PROPOSAL FOR AN ENMC WORKSHOP ON:

Clinical trial readiness for Calpainopathies

WORKSHOP ORGANISERS

Dr. Isabelle Richard, PhD (Genethon, UMR951, Evry, France) directs the Muscular Dystrophy Unit at Genethon, and is Director of Research at the CNRS. She was the first to identify the genetic basis of a limb-girdle muscular dystrophy (LGMD2A, Calpain 3) and contributed to the identification of genes or mutations in a number of other neuromuscular disorders. She is an expert in the development of animal models for muscular dystrophies and in the pre-clinical validation of gene therapy approaches. Her interests go from the molecular genetics of neuromuscular diseases to muscle physiology and the identification of normal and pathological biochemical and molecular networks. Regarding the topic of the proposed workshop, she identified the gene, developed animal models deficient in calpain 3, perform functional studies and tested an AAV-mediated transfer of calpain 3. Through these studies, she has developed a number of national and international collaborations in the calpain 3 field.

Pr. Adolpho Lopez de Munain, MD, (Biodonostia Research Institute, San Sebastian, Spain) lead the Neuroscience Area of Biodonostia Institute one of the 29 Institutes for Biomedical Research accredited by Institute Carlos III in Spain. He is also Senior Clinician at the Department of Neurology of Universitary Hospital Donostia and Associated Professor of Neurology at the University of the Basque Country. He is an active member of the Spanish Society of Neurology, where he served as coordinator of the study groups on Neuromuscular Diseases and Neurogenetics. He served as President of the Society of Neurology of the Basque Country. Currently is also an active member of the Spanish Association of Human Genetics, the World Muscle Society and Eusko Ikaskuntza (Basque Studies Society).
The common line of his work has been clinical neurogenetic research with contributions in several clinical fields of Neurology mainly in neuromuscular disorders and specifically in LGMD2A. He is author or coauthor of more than 220 peer-reviewed articles, 25 book chapters and more than 400 communications to national and international conferences and invited lectures. In this period he has been Director of 12 doctoral theses (3 over LGMD2A) on some of the exposed lines. Regarding the topics of the proposed workshop, he is involved in radiological characterization of LGMD2A in order to evaluate natural history and modifications due to therapies and the phenotype/genotype correlations in benign LGMD2A cases. He is also interested in the role of calpain 3 in the maintenance and turnover of satellite cells in skeletal muscle.

Dr. Martin Krahn, MD, PhD, is Associate Professor in Medical Genetics at the University-Hospital Center of Marseille (Assistance Publique - Hôpitaux de Marseille; Faculty of Medicine - Aix Marseille University). Since 2004, he is in charge of the genetic diagnosis of various forms of myopathies, including Calpainopathies, in the Department of Medical Genetics (Pr. N. Lévy), as part of a French national laboratory reference activity. This diagnostic activity is closely linked to research activities in the UMR_S910 Inserm-AMU research unit (Pr. N. Lévy), within the "Translational Myology" team (co-direction with Dr. M. Bartoli), covering translational research oriented towards diagnostic and therapeutic applications in the field of myopathies, including the participation to European FP7 projects (NMD-CHIP, BIO-NMD, NEUROMICS).
Regarding the topic of the proposed workshop, he has coordinated in the past years national and international translational research projects, including the analysis of large French cohorts of calpainopathies, the characterization of the *CAPN3* mutational spectrum, and the identification of *CAPN3* mutations in Eosinophilic Myositis (collab. with Pr.Lopez de Munain).

WORKSHOP JUSTIFICATION

Background:

Genetic defects in the calpain3 gene (*CAPN3*) lead to the autosomal recessive limb girdle muscular dystrophy 2A (LGMD2A; OMIM 253600), also called calpainopathy [1]. It was first identified in a population from the Réunion Island in the Indian Ocean and later shown to be one of the most frequent LGMD with reported prevalence between 10 to 70 per million [2-4]. The deficiency results in a slowly progressive muscle disorder with onset between the first and second decade of life and a mean age of loss of independent ambulation after about 15 years of evolution. Muscle weakness is remarkably symmetrical and predominant in the axial muscles of the trunk and proximal muscles of the lower limb [5]. A large number of patients have a reduced forced vital capacity but although respiratory complications are possible, it is not salient feature in primary calpainopathy. There is no cardiac involvement and when reported is likely to be coincidental[5, 6].

Calpain3 is a member of the calpain family of non-lysosomal calcium-activated cysteine proteases. Although some isoforms were identified in lens and brain, the classical full-length form of *CAPN3* is primarily expressed in skeletal muscle [7]. Expression in the heart has also been reported during development and in murine and human adult tissues at a level 100 times lower than in skeletal muscle [8 9]. In muscle, the enzyme is present as a dormant enzyme probably through interaction with one of its partner, the giant protein titin [10 11]. It was shown that calpain 3 self-activates by autolysis through the removal of an internal peptide to free the catalytic site for substrate accessibility[12, 13]. The precise function(s) of calpain3 and the mechanism by which it causes LGMD2A are not fully understood although several evidences pinpoint a role in cytoskeleton remodeling[14-16].

To date, there is still no cure for the disease. In an attempt to develop a therapeutic strategy based on gene transfer for LGMD2A, we have initiated a series of experiments using recombinant Adeno-Associated Viral (rAAV) vectors, the current standard tool for gene transfer in skeletal muscle. After local injections of rAAV vectors expressing *CAPN3* in

skeletal muscle of a murine model deficient in calpain 3, we showed an efficient *CAPN3* transgene expression in skeletal muscles with a correct localization at the sarcomere [17]. This expression was associated with a restoration of the calpain3 proteolytic activity and rescue of the contractile force deficits. However, when testing a systemic route of administration of these vectors, a cardiac toxicity was detected and confirmed to be related to ectopic expression and unregulated activity of calpain 3 in the heart [18]. A second generation of vectors was then designed using new promoters and by introducing a sequence target of a cardiac specific microRNA (miR-208a) in the 3'UTR. These modifications suppress the cardiac toxicity while conserving the therapeutic effect in the skeletal muscles [18]. Following these studies, we have now conducted a preclinical study in normal macaca fascicularis to evaluate the biodistribution and safety of AAV9-calpain 3 vectors with a deep analysis of the heart. In conclusion, with these studies, we were able to demonstrate the safety of the calpain 3 vectors and to define a dose that showed a therapeutic benefit, supporting the use of AAV-mediated transfer of the calpain 3 gene in clinical studies in LGMD2A patients.

References:

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Aims and objectives of the workshop

The promising results obtained from the AAV-mediated transfer of the calpain 3 gene in animal studies, paves the way for a gene transfer clinical trial in humans with Calpain 3 deficiencies. The aim of this workshop is to assemble worldwide experts in the fields of calpainopathies, to brainstorm on the feasibility of such a clinical trial and the steps needed. Covered topics will be natural history of the disease, patient registries, update on gene transfer results, alternative therapies and biomarkers/outcome measures. This workshop will also be the first step to construct a network of experts that potentially will be involved in future clinical trials. The workshop will help to pave the way towards putting the AAV product into clinical trials.

From a practical point of view, William Lostal will be writing notes, the lay report will be written by Isabelle Richard, Adolfo Lopez de Munain and Martin Krahn and the discussions will be led by Adolfo Lopez de Munaim, Andoni Urtizberea, Martin Krahn and Isabelle Richard.

- **WORKSHOP DELIVERABLES**
  - To review the clinical phenotype of LGMD2A patients and the need for further characterization of Natural History in Calpain 3 deficiencies
  - To evaluate the state of patients registries
  - To review the current state of outcome measures and biomarkers

- To organize a network of clinicians for future clinical trials

- **TIMING OF THE WORKSHOP**

Fall 2017

Rationale: Results obtained by AAV-mediated transfer of C3 on mouse models are promising. In the perspective of moving this strategy to clinical trial, there is an urgent need to compile patient data and establishing a formal European/international consortium of clinicians for developing and performing clinical trials

PROPOSED WORKSHOP PROGRAM:

Friday, 1:30PM	Introduction, Workshop Overview	ENMC representative; organizers
	Session 1: **LGMD2A overview,**	Chair: Isabelle Richard
2:30-3:00	History of the identification of LGMD2A	Michel Fardeau
3:00-3:30	Clinical presentation of calpainopathies	Adolfo Lopez de Munain
3:30-4:00	The calpain family	Hiroyuki Sorimachi
4:00-4:30	*Break*	
	Session 2: Patient landscape, registries and database, patient foundation	Chair: Marin Krahn
4:30-4:50	LGMD2A in France	Bruno Eymard, Guilhem Sole
4:50-5:10	LGMD2A in Spain	Adolfo Lopez de Munain, Jorge Díaz-Manera
5:10-5:20	LGMD2A in UK	Michela Guigleri
5:20- :30	LGMD2A in Germany	Maguy Walter
5:30-5h50	LGMD2A in Italy	Claudio Semplicini , Marina Fanin
5:50:6:00	LGMD2A in east europe	Lenka Fajkusová,
6:00-6:15	Dominant Calpainopathies	John Vissing
7:00-9.00	*Dinner*	
Saturday	**Session 2: (part II)**	Chair: Andoni Urtizberea
9:00-9:30	Calpainopathies outside Europe	Vincent Carson, Mayana Zatz
9:30-10:00	Patient registries	Michela Guigleri
10:00-10:30	Patient organisation/ C3	Jenifer Levy
10:30-11:00	Patient representative	Bruno Kullman
11:00-11:30	*Coffee break*	
	Session 3: Outcomes measures	Chair: Adolfo de Munain
11:30-12:00	Natural history of LGMD2A	Andoni Urtizberea
12.00-12.30	Respiratory aspects	Helene Prigent
12:30-13:00	Outcomes measures: AIM experience	Jean-Yves Hogrel
1.00-2:00 PM	*Lunch*	
2:00-2:30	Imaging profiles	Robert-Yves Carlier
2:30-3:00	Regulatory aspects for clinical trials	Geraldine Honnet
3:00-3:30	*General discussion on steps towards a clinical trial*	All

3:30-4:00	*Break*	
	Session 4. Function, models and therapies	Chair: Jenifer Levy
4.00-4.30	Animal models	William Lostal
4:30- 5:00	AAV gene transfer	Isabelle Richard
5:00-5:30	C3 and SERCA	Adolfo Lopez de Munain
5:30 – 6:00	Calpain 3 biochemical function	Hiroyuki Sorimachi
7:00-9:00	*Dinner*	
Sunday	**Session 5: Workplan**	Isabelle Richard, Martin Krahn, Adolfo Lopez de Munain, Andoni Urtizberea
9:00-10:00	Defining the key points of action	
10:00-11:00	Plan for next tasks and timetable	
11:00-11:30	Conclusions and lay report	

PROPOSED LIST OF PARTICIPANTS:

(The persons indicated by an asterisk have already agreed to come.)

1. ***Isabelle Richard,** Genethon, Evry, France. richard@genethon.fr Researcher on pathophysiology and therapy of LGMD2A
2. ***Adolfo Lopez de Munain**, Biodonostia Research Institute, San Sebastian, Spain. ADOLFO.LOPEZDEMUNAINARREGUI@osakidetza.net Neurologist, specialist of LGMD2A
3. ***Martin Krahn** Faculté de médecine La Timone, Marseille, France. martin.krahn@univ-amu.fr. Diagnosis of LGMD2A
4. ***Robert-Yves Carlier** Hôpital Raymond Poincaré, Garches, France. robert.carlier@aphp.fr Imaging by MRI. Responsible of LGMD2A withing the MYO-MRI project.
5. ***Bruno Eymard,** AIM-Hôpital Pitié-Salpêtrière, Paris, France. anne-marie.maronne@aphp.fr Neurologist.
6. **Lenka Fajkusová**, University Hospital, Brno, Tcheque Republicc. lenka.fajkus@gmail.com Neurologist
7. ***Michel Fardeau,** Hôpital Pitié-Salpêtrière, Paris, France. Neurologist. m.fardeau@institut-myologie.org Discovery and description of LGMD2A
8. ***Michela Guigleri,** Institute of Human Genetics, Newcastle, UK. michela.guglieri@newcastle.ac.uk Neurologist
9. ***Jean-Yves Hogrel** Hôpital Pitié-Salpêtrière, Paris, France. Jean-Yves HOGREL jy.hogrel@institut-myologie.org Specialist on outcomes-measures
10. ***Geraldine Honnet**, Evry, France Genethon, Evry, France. ghonnet@genethon.fr Clinical development expert.
11. ***Jennifer Levy-keiser,** C3 foundation, USA. jennifer@curecalpain3.org Foundation representative.
12. ***Jorge Díaz-Manera**, Hospital de la Santa Creu i Sant Pau, Barcelone, Spain. JDiazM@santpau.cat Neurologist

13. **Helene Prigent,** Hôpital Raymond Poincaré, Garches, France. helene.prigent@aphp.fr Pneumologist
14. ***Claudio Semplicini**, University of Padova, Padova, Italy. claudio.semplicini@unipd.it Neurologist
15. ***Guilhem Sole** CHU Bordeaux, Pessac, France. guilhem.sole@chu-bordeaux.fr Neurologist
16. **Hiroyuki Sorimachi,** Univ. Tokyo, Japan. sorimachi-hr@gakuken.jp Research expert in calpains.
17. ***Andoni Urtizberea**, Hôpital Marin - APHP, Hendaye, France. andoni.urtizberea@hnd.aphp.fr Neurologist
18. ***John Vissing,** University of Copenhagen, Copenhagen, Denmark. vissing@rh.dk Neurologist
19. **Maguy Walter** Friedrich Baur Institute, Munich, Germany. maggie.walter@lrz.uni-muenchen.de Neurologist
20. **Mariz Vanhof**
21. **Vincent Carson**, Clinic for Special Children, Strasburg, US
22. **Marina Fanin**, University of Padova, Italy. marina.fanin@unipd.it.
23. **Bruno Kullman**, patient representative. Italy

Young researchers

William Lostal, Genethon, Evry, France. Lostal@genethon.fr